Co-localization of RGD and PHSRN peptides on titanium to guide stem cell behaviour and enhance Implant osteointegration

Roberta Fraioli[1,2,3], José María Manero[1,2], Javier Gil[1,2], Carlos Mas-Moruno[1,2,3]

[1]Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Metallurgical Engineering, Technical University of Catalonia (UPC), Spain
[2] Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Campus Río Ebro, Spain
[3] Centre for Research in NanoEngineering (CRNE), Universitat Politecnica de Catalunya, Spain

roberta.fraioli@upc.edu

INTRODUCTION

Controlling the interface between the implant and the surrounding tissue is of paramount importance to ensure the success of implantable devices. Adverse body reactions to the implant, such as fibrous encapsulation, can be avoided by accurately tuning the properties of the synthetic material surface. For this purpose, one approach, which is explored in this study, is to chemically functionalize the surface with bioactive molecules, capable of binding cell receptors and thereby influence cell behaviour[1]. Specifically, the aim of our modification is to guide the response of human mesenchymal stem cells (hMSCs), enhancing adhesion, growth and differentiation into the osteoblastic lineage. Following a biomimetic approach, the two fibronectin fragments RGD and PHSRN have been chosen as bioactive moieties of our double-branched ligand (fig. 1). This recently synthesized peptidic platform[2] guarantees the co-presentation of the motifs, which are known to synergically bind integrin α5β1[3], a key receptor in osteogenesis[4].

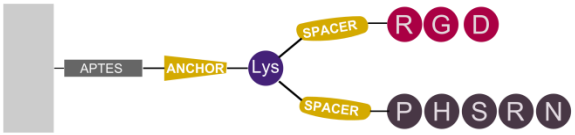

Fig 2. Schematic representation of the dual ligand for the co-presentation of RGD and PHSRN sequences.

EXPERIMENTAL METHODS

Surface functionalization and characterization

The divalent platform was covalently immobilized on smooth mirror-like commercially pure Ti (grade 2) via silanization with 3-(aminopropyl)-triethoxysilane (APTES). The physicochemical properties of the surface were studied with contact angle measurement, white light interferometry, and XPS.

MSC adhesion, growth and differentiation

The response of hMSCs was analyzed by means of immunofluorescence, proliferation and differentiation studies. These included evaluation of calcium deposits formation by Alizarin Red staining method, analysis of ALP expression via enzymatic-assay, and gene expression by RT-PCR.
One-way ANOVA with Tukey post-hoc analysis was used to detect significant differences.

RESULTS AND DISCUSSION

The immobilization of the dual ligand significantly increased the adhesive capacity of the metallic surface: more cells attached to the surface in serum-free conditions and their projected area is significantly increased (fig. 2). Proliferation of cells was efficiently supported by the synthetic ligand at all time points. Analysis of calcium deposits, ALP activity and gene expression revealed that the branched ligand is able to support the differentiation into the osteoblastic lineage. This study provides further evidence that an accurate choice and controlled presentation of peptidic ligands is crucial to design bioactive coatings and

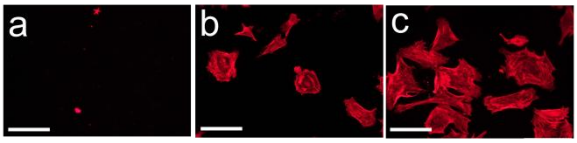

Fig 1. Actin immunostaining of the uncoated (a), RGD-functionalized (b), and RGD/PHSRN dual ligand-functionalized (c) Ti disks. Bar 500 µm in (a), 200 µm in (b) and (c).

significantly improve the biological performance of an implantable material.

CONCLUSION

The covalent immobilization method was proved efficient to stably anchor the peptidic ligand to the metallic surface. Stem cells attached and spread to a higher extent on the surfaces coated with the dual biomolecule, displaying a polygonal shape, which is associated to the differentiation into bone-forming cells. The analysis of gene expression, ALP activity and calcium deposits also confirmed that the simultaneous co-presentation of the RGD and PHSRN motifs is able to stimulate the differentiation of stem cells into osteoblasts. Hence, our functionalization strategy is proved to be a promising and versatile approach to guide stem cells behaviour by tailoring surface anchored ligands.

REFERENCES

1. Maia F. R. *et al.*, Acta Biomater. 9:8773-8789, 2013
2. Mas-Moruno C. *et al.*, ACS Appl. Mater. Interfaces. 6:6525-6536, 2014
3. Aota S. *et al.*, J. Biol. Chem. 269:24756-24761, 1994
4. Martino M. M. *et al.*, Biomaterials 30:1089-1097, 2009

ACKNOWLEDGMENTS

RF and CMM thank the Generalitat de Catalunya and the Government of Spain for financial support (SGR2009 1039, MAT2012-30706). CMM also thanks the European Commission (FP7-PEOPLE-2012-CIG).